Title
Temporal impact of sugar metabolism on the liver

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Temporal impact of sugar metabolism on the liver

by
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Temporal impact of sugar metabolism on the liver

Ravi Chachad

ABSTRACT

Purpose: Increased sugar consumption is associated with metabolic conditions that can result in poor health outcomes. To investigate the impact of sugars such as glucose and fructose in the body, this study aims to determine the timing of effects and assess the impact of these effects. This study aims to demonstrate acute effects in metabolism as a result of sugar metabolism.

Methods: Six male participants were imaged on a 3T MRI scanner at a fasted state then subsequently every hour for up to eight measurements. Between scanning sessions, they consume a ^{13}C labeled glucose or fructose shake, have breath collected, and have blood drawn; this is repeated on a separate day to satisfy the other experimental condition. The MRI exam consists of Proton Density Fat Fraction (PDFF), proton Magnetic Resonance Spectroscopy (^{1}H MRS), and ^{13}C MRS. The images are processed to analyze liver volume and the spectra from the MR Spectroscopy are normalized and the peaks are quantified.

Results: Liver volume is significantly different from baseline measurements at 2-, 3-, and 4-hours post-feeding with p=0.032, p=0.003, and p=0.009 respectively. Fat content is significantly different from baseline measurements at 3- and 4-hours post-feeding with p=0.026 and p=0.048 respectively. Different MR measures of fat fraction in the body produce a significant positive correlation (p=0.003). The median change of lipids (CH_2) positively correlates with the median change in glycerol (p=0.011). Fat fraction does not significantly correlate with the blood measures taken, but when high choline and low choline groups are separated, new formed lipids in the blood and long-term storage of fat differ significantly, p=0.021 and p=0.03 respectively.

Conclusions: Choline classification of participants resulted in a difference in new lipids in the blood and long-term storage in the liver. Low choline individuals tended to export less lipids in the blood and store more in the liver. High choline individuals exported more lipids and retained less in the liver. MR exams of the liver evaluate the health of the liver and burden to abdominal organs, whereas blood collection provided a glimpse into cardiovascular burden.

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1 INTRODUCTION

Sugars are consumed daily in a variety of foods. Increased consumption of sugars has been shown to incur deleterious health effects, promoting obesity, steatosis, diabetes and cardiovascular disease, while a reduction of sugars has been shown to have beneficial metabolic effects [1]. Two of the most commonly consumed sugars are glucose and fructose. Both are metabolized by the body in different ways; glucose is metabolized in various parts of the body and fructose is solely metabolized by the liver. Fructose has been suggested to be particularly damaging, in part due to its almost exclusive hepatic metabolism [2]. Fructose metabolism in excessive quantities can result in diabetes, obesity, and other health risks [3]. This can contribute to the increase of nonalcoholic fatty liver disease (NAFLD) which is proven to be associated with cardiovascular and metabolic complications [4]. Glucose when consumed stimulates insulin production that in causes cells to uptake glucose. In the liver, glucose is broken down into carbon dioxide and water, turned into glycogen, or converted in a series of other processes. Fructose undergoes processes where it can be broken down in cellular respiration, converted into glycogen, or used in the formation of fats. Fat accumulation in the liver can result in steatosis and a variety of other conditions that are associated with poor health outcomes and can be measured with Magnetic Resonance Imaging (MRI). MRI scanners manipulate a magnetic field in order to produce structural and functional images. Different tissues have different relaxation times which result in contrast to discriminate different physical structures. Fat can be discriminated in MRI and can be calculated using post-processing. Fats can also be exported out of the liver in the form of triglycerides and very-low density lipoprotein (VLDL). Triglycerides can be freely exported out of the liver and into the bloodstream whereas, VLDL is packaged in a vesicle. These vesicles are composed of the cell membrane which have phosphatidylcholine and

other phospholipids. To capture the metabolic fates of ingested fructose and glucose, blood measures can help quantify excreted fats, packaged as lipoproteins [5].

The purpose of the study was to evaluate the temporal effects of consuming glucose and fructose in the fasted state and determine their metabolic fates. To further studies to elucidate the roles of fructose and glucose, there is a need to understand their different metabolism and the timing of effects post consumption. Stable isotope labeled glucose and fructose can enable measurement of their metabolites with MR measures of liver volume providing indirect, further evidence of liver metabolism. Therefore, the goal of this study is to determine the timing and size of the metabolic fates of glucose and fructose post-feeding. The approach of this study is to evaluate subjects in a fasted state, then hourly, post-feeding with stable isotope labeled shakes, measuring excreted CO_2, liver volume, liver fat fraction, ^{13}C MR measured liver lipids, and lipoprotein measures in the blood.

2 METHODS

Six, non-diabetic male participants, who had fasted overnight, were studied on two separate days. Demographics and some body metrics related to body composition and insulin kinetics can be found in Table 1. This study measures the main metabolic fates of glucose and fructose in the breath, liver, and blood. The fates of interest are carbon dioxide, glycogen, triglycerides, and VLDL.

Table 1: Demographics of study participants. Fat free mass and fat percentages were determined by Dual Energy X-ray Absorptiometry (DEXA).

Participants (n=6)	**Mean ± SD (Percentage)**
Age (yrs)	35.66 ± 15.95
Weight (kg)	110.54 ± 17.29
Body Mass Index (Kg/m^2)	36.03 ± 4.32
Fat Free Mass (%)	67.82 ± 8.51
Fat (%)	38.00 ± 6.14
Homeostatic model assessment of Insulin Resistance (HOMA-IR)	6.03 ± 3.57

2.1 Study day

One day participants received ^{13}C-glucose labeled shakes after their fasted, baseline measures and repeatedly throughout the day and the other day they received ^{13}C-fructose labeled shakes on the same schedule (Figure 1). The hourly measures taken include: CO_2 collection via breath, Fat fraction (FF) by MR Spectroscopy (MRS), FF by Proton Density Fat Fraction (PDFF), liver volume based on PDFF images, fat and potentially other metabolites via 13C MRS, collection and DNL/VLDL production via blood draws and mass spectrometry analyses [6]. Each cycle lasts about one hour and the 3T MRI exam takes about 30 minutes to complete.

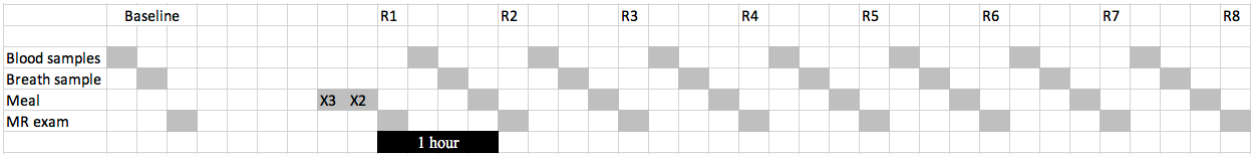


Figure 1: Study day diagram with all measures.

2.2 Carbon dioxide breath collection

Carbon dioxide was collected after consuming the labeled shake. Participants exhaled into a vial using a straw which they slowly pulled out of the vial. Once the straw was no longer in the vial, a researcher quickly closed the vial and stored it for future analysis of the ^{13}C enrichment of the expired CO_2 which was labeled with, performed off-site at Metabolic Solutions, Inc. (Nashua, New Hampshire).

2.3 Proton Density Fat Fraction (PDFF)

Proton density fat fraction images were obtained through the liver (10mm slice thickness), using the Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation (IDEAL) sequence acquired in one breath hold at end inspiration.

2.4 Liver segmentation

Liver volumes were semi-automatically generated using an in-house, previously developed method incorporating the multiple images from the IDEAL sequence (R2*map, In-phase image, and fat and water images). Automatic results sometimes included extrahepatic tissue, particularly if the subject had low levels of fat in the liver. These regions were manually identified and trimmed from the liver ROIs such as where the liver extended into the ribs, extended into the kidney, and included the heart and stomach (Figure 2).

a) b)

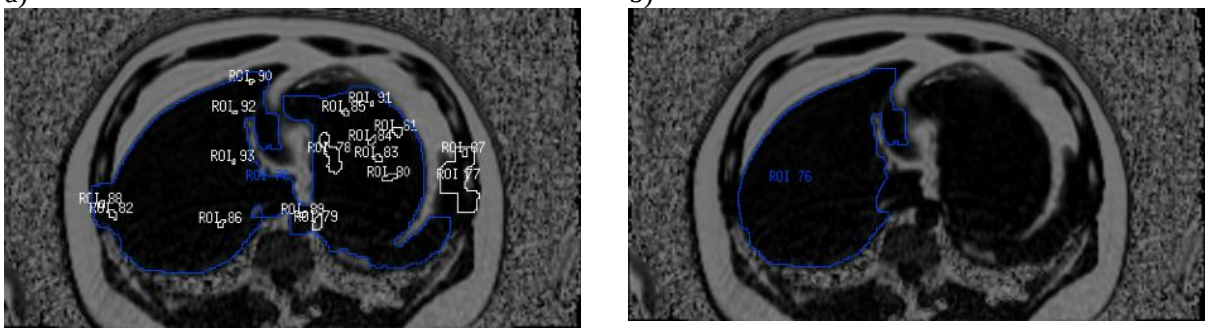


Figure 2: Semi-automatic liver segmentation. a) Blue region indicates area perceived to be the liver after automatic segmentation is performed. b) After allowed manual edits are performed, the liver volume is corrected.

2.5 MR Spectroscopy (^{1}H MRS)

Proton spectroscopy was obtained from a 20cc voxel in the liver, with TR/TE = 2500/30. Spectra were automatically phase-, frequency-, motion- and T2 relaxation-time corrected [7]. MR liver fat fractions were calculated from the corrected MR measures of CH_2 and CH_3 lipids and of water as the total lipids divided by total lipids and water. From the same spectra, a choline peak was quantified by calculating area under the peak and normalizing to the total lipids and water peak areas.

2.6 Carbon-13 MR Spectroscopy (^{13}C MRS)

2.6.1 Acquisition

^{13}C MR spectroscopy was obtained using an in-house built receiver coil tuned to the ^{13}C resonant frequency, placed on the patient's right abdomen. Data was acquired both from a

selective pulse, localized to an oblique coronal plane (parallel to the coil) through the liver, excluding the subcutaneous fat. A selective pulse was used to acquire 384 acquisitions with 4096 points and 10,000 Hz from a 40mm slab through the liver.

2.6.2 Processing

The ^{13}C spectroscopy data was processed by performing an apodization and creating a level baseline. Under the long chain CH_2 and CH_3 lipid peak, there were broad peaks. These peaks were estimated using the points from both sides of the tails, outside the range of the higher, narrow peaks, to fit two Gaussian curves. The CH_2 peak was used as the reference for all subsequent peak locations and was defined at 30.41ppm. The area of the fit for the CH_3 peak had an amplitude limited to one-tenth that of the CH_2 peak. These curves were subtracted to produce a flat baseline and to normalize data to compare between timepoints. An automatic method was also applied to the ^{13}C MR spectra to identify the peaks of interest and report the area under the curve (Figure 3).

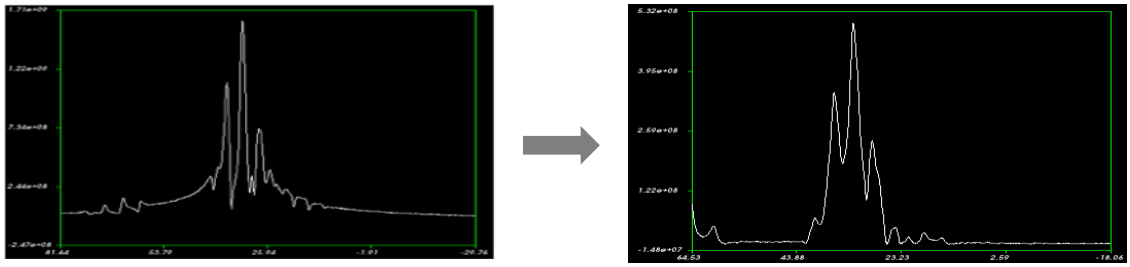


Figure 3: ^{13}C MRS Baseline Correction. Starts with the processed data (left), then fit two broad peaks. Subtract the broad peaks from the processed data and receive a spectrum with a new baseline (right).

2.6.3 Quantification

Peaks are quantified by taking the area under the curve. The peaks of interest in this study were lipids (CH_2) and glycerol (GLC).

2.7 Blood draws

Blood draws were conducted hourly and were processed off-site at Touro University (Vallejo, California) using mass spectrometry. Blood is separated into four different samples: gold (8cc), grey (2cc), red (4cc), and purple (7cc). Gold samples are in serum-separating tubes (SSTs). Grey samples are processed with sodium fluoride and potassium oxalate. Red is processed with perchloric acid, and purple is processed with K2 EDTA. TG-Rich Lipoprotein (TRL) and VLDL were analyzed for percent DNL in those associated lipid compartments. Fractional DNL and precursor pool enrichments were measured by stable-isotope GC/MS-MIDA methodology [2].

2.8 Overall Analyses

Measures at each timepoint were compared to baseline to determine any significant differences. Data was evaluated as change or percent change from baseline. Time courses were plotted to evaluate the change over the course of the day and significance of timepoints were determined. Different measures were correlated to identify connections between the multiple measures collected. Statistical significance was determined by $p<0.05$. Statistical tests performed include Mann-Whitney U test, Cohen's D for effect sizes, paired sample t-test, Pearson's correlation, and Kendall correlation. Data when analyzed as a complete set can be analyzed using parametric tests due to the power reinforced by the number of measurements. When subletting data, non-parametric tests are used. Correlations for both a normal distribution and non-normal distribution were performed, Pearson correlation and Kendall correlation respectively.

3 RESULTS

3.1 Carbon dioxide through the day

Exhaled carbon dioxide was collected post ingestion and the labeled carbon-13 carbon dioxide was quantified (Figure 4). The fructose day and glucose day carbon dioxide differed as more label appeared in the carbon dioxide produced on the fructose day when compared using a paired t-test of all the timepoints (p=0.0006). From the current data, when comparing fructose and glucose days, it can be anecdotally observed that carbon-13 that is incorporated into lipoproteins in the blood is detected earlier on the fructose day than on the glucose day.

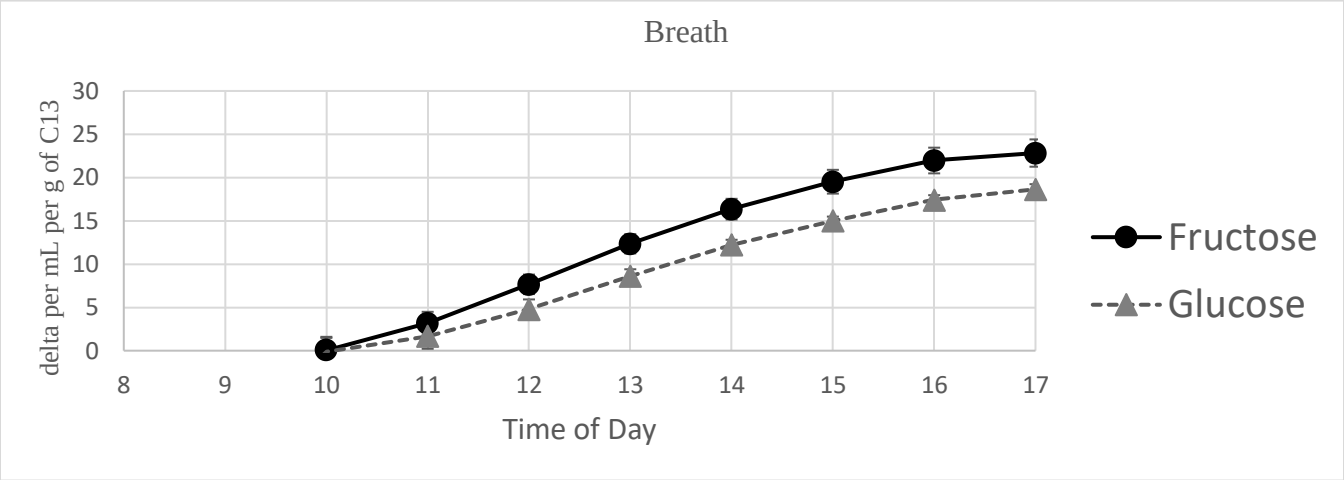


Figure 4: Change of labeled carbon dioxide exhaled in the breath.

3.2 Liver volume through the day

For each participant, there was not a significant difference in their quantified liver volume on each study day (p=0.95). Due to the range of liver volumes in the study sample, liver volume was compared as a ratio to baseline (Figure 5). Some points through the day have a significantly different measurements than at baseline (Table 1).

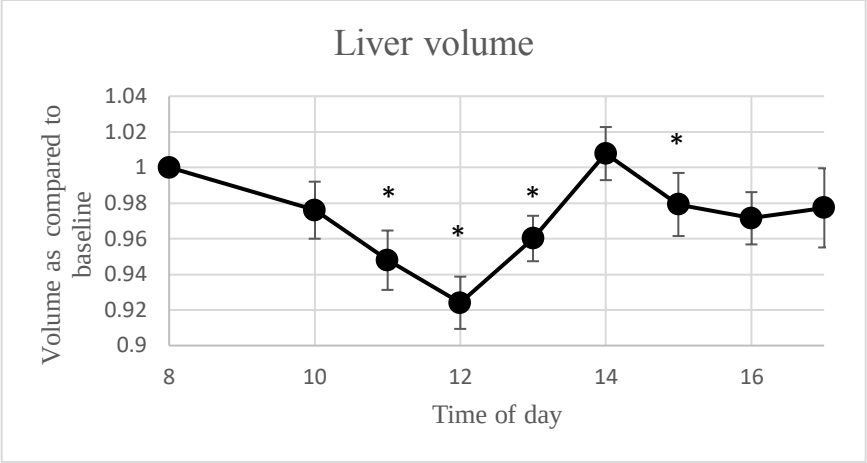


Figure 5: Liver volume as a compared to baseline. Data is reported as median ± SE (*p<0.05).

After using the semi-automatic method to obtain the liver volume, all the ROIs that compose the slices of the liver were analyzed. The fat fraction was the fat divided by fat plus water as generated by the scanner in the PDFF. To reduce the influence from noise or error due to visceral fat being segmented, the median fat fraction was used. Comparing the time course of fat fraction is a rough estimate of fat change throughout the course of the day as compared to baseline (Figure 6).

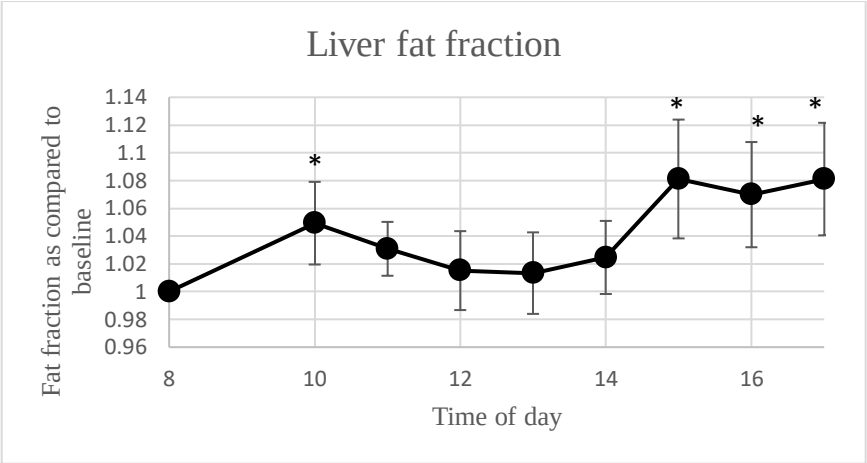


Figure 6: Liver fat fraction as compared to baseline. Data is reported as median ± SE (*p<0.05).

Total fat content is the product of liver volume and fat fraction. Comparing the time course of fat content is another estimate of fat change throughout the course of the day as

compared to baseline (Figure 7). This is a measure of how much fat is in the liver considering the amount of fat and water. The statistical significance between each time point through the day and baseline is listed in Table 2.

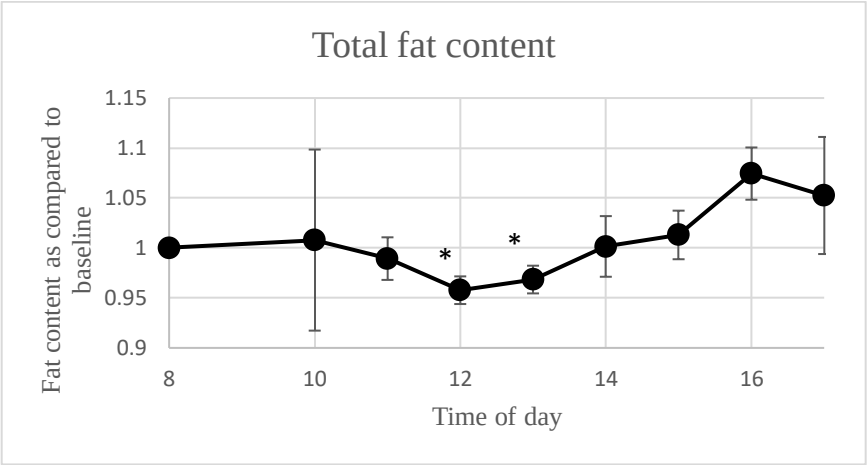


Figure 7: Liver fat content as a compared to baseline. Data is reported as median ± SE (*p<0.05).

Table 2: Ratio of liver volume, fat fraction, and fat content in comparison to baseline. (*p<0.05, **p<0.01).

8 am (Baseline) vs	Liver volume	Fat fraction	Fat content
10 am (r1)	0.976	1.049*	1.008
11 am (r2)	0.948*	1.031	0.989
12 pm (r3)	0.924**	1.015	0.957*
1 pm (r4)	0.960**	1.013	0.968*
2 pm (r5)	1.007	1.025	1.002
3 pm (r6)	0.979*	1.081*	1.013
4 pm (r7)	0.971	1.070*	1.074
5 pm (r8)	0.977	1.081*	1.052

3.3 Fat fraction comparison

Fat fraction is generated in the PDFF scans and through drawing ROIs encompassing the volume of the liver, the fat fraction of the liver is calculated. From the proton MRS, a fat fraction

is also calculated. These two values should correlate if the fat fraction in the voxel is representative of the fat fraction in the entire liver (Figure 8).

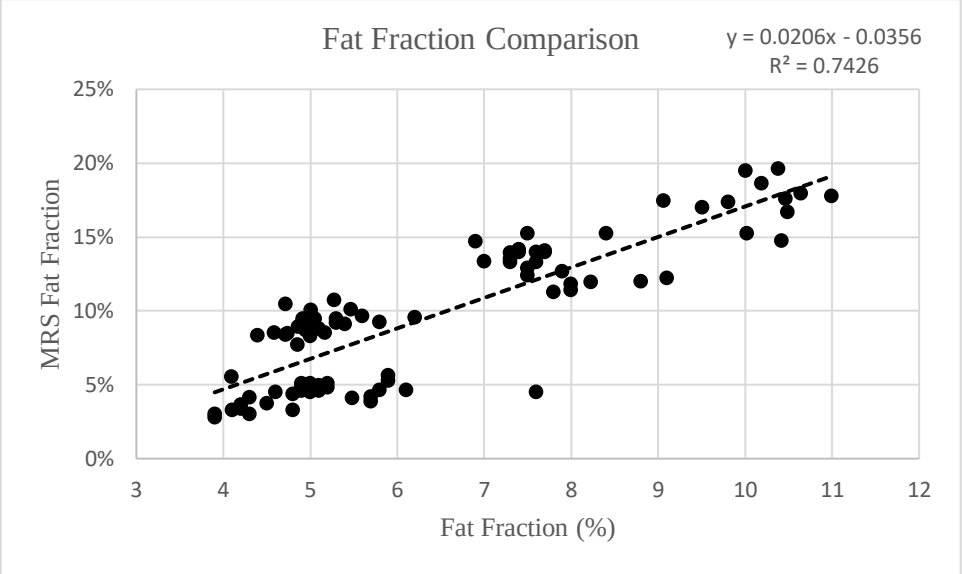
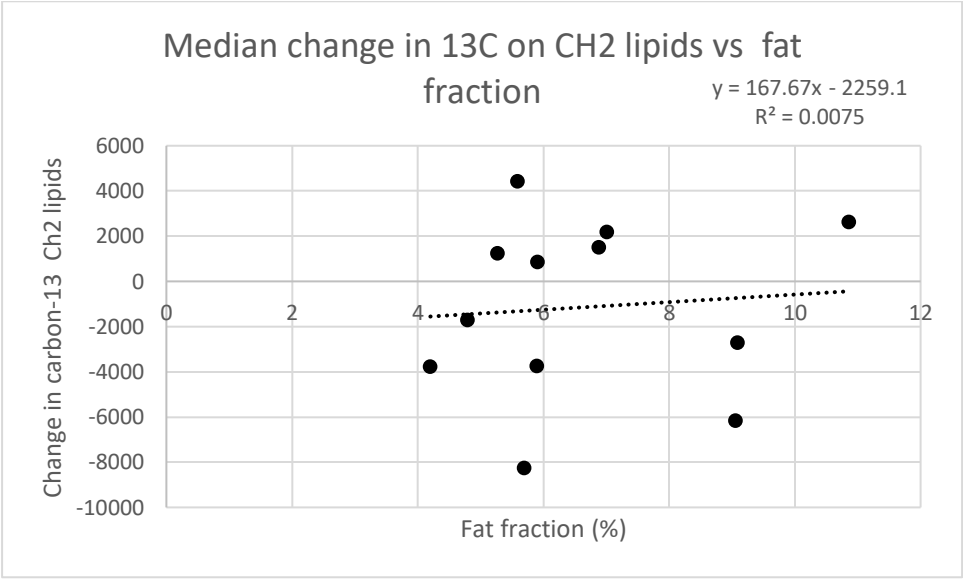


Figure 8: Comparison of MRS fat fraction and calculated fat fraction from PDFF

3.4 Composition of fat stored in the liver

CH_2 lipids at the end of the day do not correlate with PDFF percentage while glycerol at the end of the day or with PDFF percentage (Figure 9).

a)



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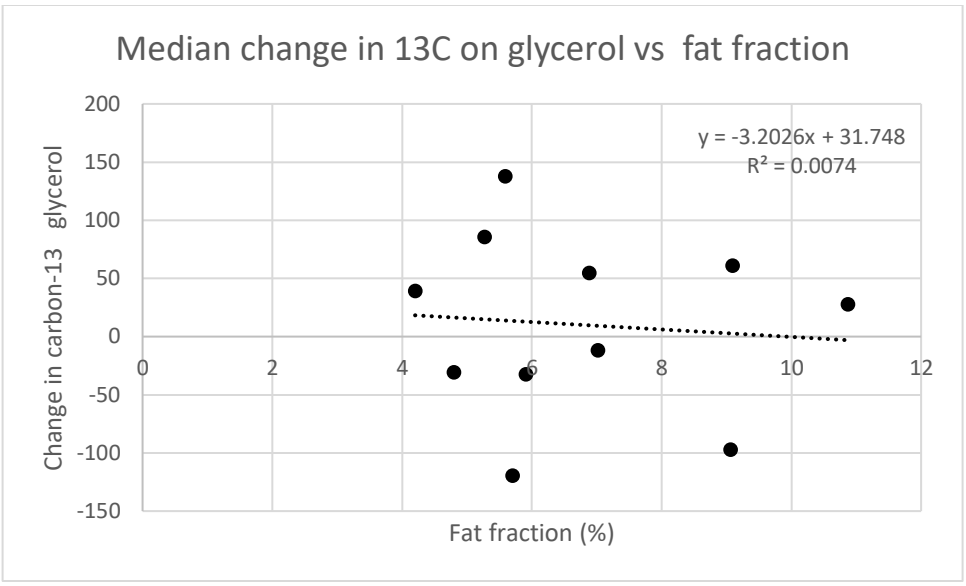


Figure 9: Fat storage in the liver at the end of the day. in a) long hydrocarbon chains and b) glycerol molecules.

Median change in glycerol from the baseline to the end of the day is positively correlated with median change in long chain hydrocarbons from the baseline to the end of the day (Figure 10).

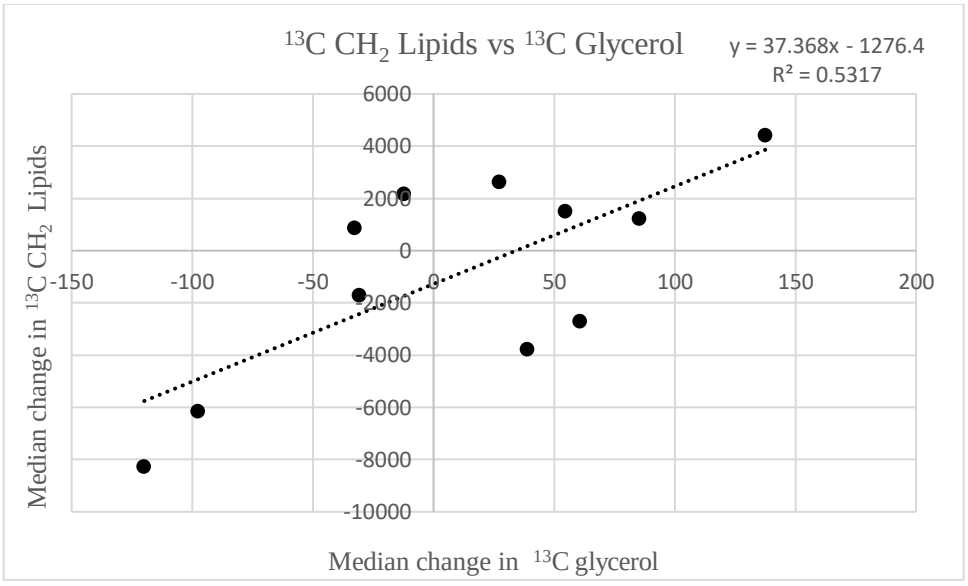


Figure 10: Correlation of median change of lipids. Lipid levels change over the course of the day as a result of feeding.

3.5 Timing of newly formed ^{13}C labeled lipids in the blood

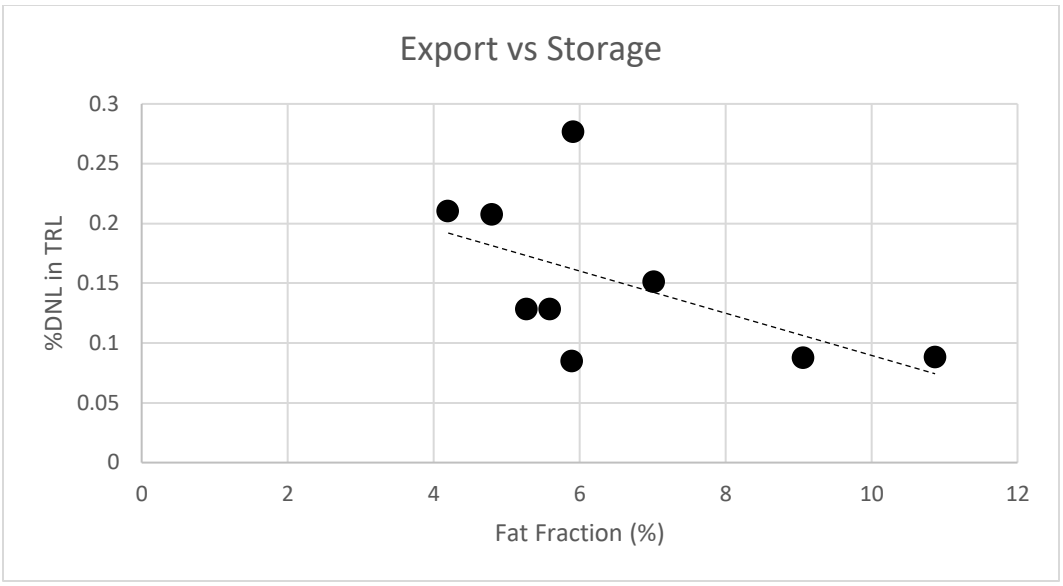
The first time at which lipids were detected in the blood from the mass spectrometry are noted in Table 4 along with time of maximum liver volume, maximum fat content, maximum glycerol amount, and maximum CH_2 lipids. The blood measure noted was %DNL in VLDL. Participants with less than 3 blood samples are excluded from this table. There are no significant correlations between any of these factors and first quantification of blood sample.

Table 3. Timing of blood and liver effects.

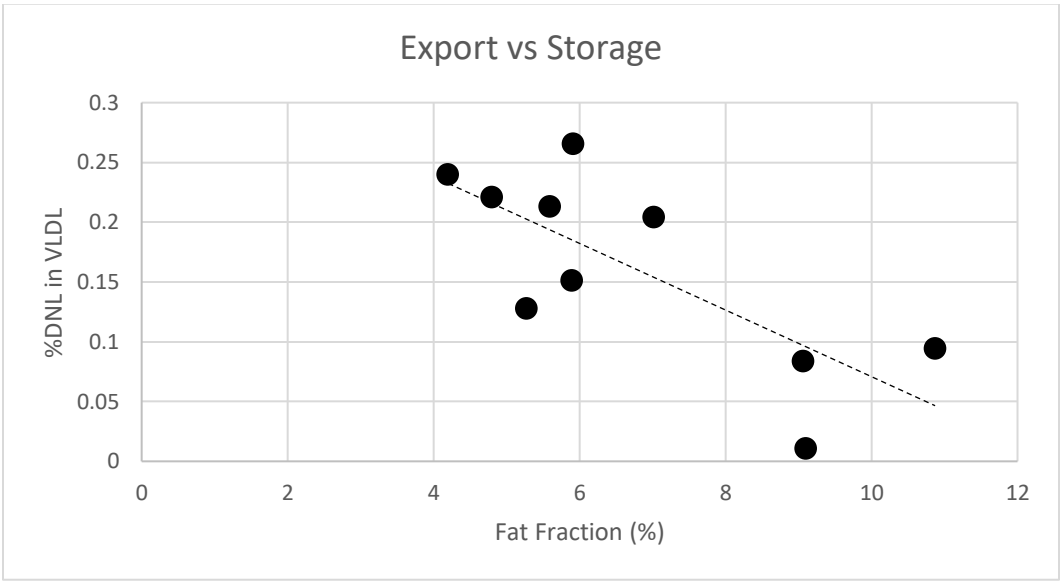
Time of maximum liver volume	Time of maximum fat content	Time of maximum glycerol amount	Time of maximum CH_2 amount	Time of first quantification of blood sample
8 am	11am	10 am	5 pm	12 pm
8 am	10 am	12 pm	5 pm	2 pm
5 pm	5 pm	11 am	10 am	11 am
5 pm	5 pm	4 pm	10 am	2 pm
8 am	12 pm	2 pm	2 pm	1 pm
8 am	4 pm	4 pm	5 pm	11 am
11 am	5 pm	10 am	8 am	12 pm
5 pm	4 pm	11 am	11 am	2 pm

3.6 Export versus Storage

TRL and VLDL are both exported by the liver out into the body in an inverse relation to how much fat the liver itself did store; fractional DNL in these lipids are displayed in Figure 11. There is a moderate negative correlation (Pearson's R^2=0.32) between long-term fat storage and TRL and also with moderate negative correlation (Pearson's R^2=0.56) between long-term fat storage and VLDL.



a)



b)

Figure 11: Correlation between fat fraction as a measure of long-term storage and lipid export into the blood. a) measured in TRL and b) measured in VLDL

The choline level of a participant is associated with their level of fat export and fat storage (Figure 12). Choline categorization of newly formed lipids in the blood is significant ($p=0.021$) as is the long-term storage of fats ($p=0.03$). In the Mann-Whitney U-test, $U<5$ is defined as significant; when grouped by high or low choline, newly formed lipids return a U-

value of 1 and long-term fat storage returns a U-value of 4. A U-value indicates that values in one sample are larger than the other.

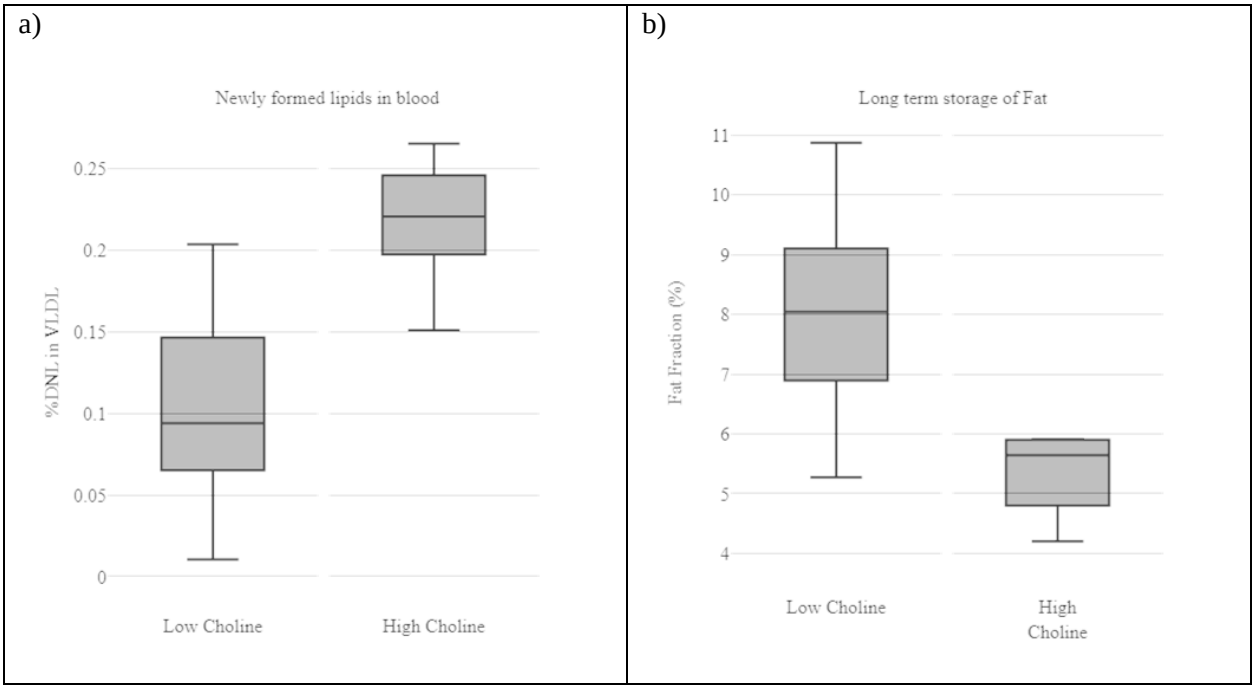


Figure 12: Choline grouping to characterize storage and export of lipids. Choline is a factor in determining the fate of lipids in the body where a) exhibits the export component and b) exhibits the storage component.

3.7 Correlations

The strength of correlations in previous sections is reported in Table 2.

Table 4: Strength and significance of correlations. *p<0.05

Variables correlated	Correlation test	Correlation Coefficient	p-value
Fat fraction vs MRS fat fraction	Pearson correlation	0.861	0.003*
Fat fraction vs median change in ^{13}C CH_2 lipids	Pearson correlation	0.009	0.78
Fat fraction vs median change in ^{13}C glycerol	Pearson correlation	0.009	0.80
Median change in CH_2 lipids vs Median change in glycerol	Pearson correlation	0.729	0.011*
Fat fraction vs %DNL in TRL	Kendall Correlation	0	1.000
Fat fraction vs %DNL in VLDL	Kendall Correlation	-0.0667	0.861
Choline vs Proton Density Fat Fraction	Kendall Correlation	-0.2121	0.381

4 DISCUSSION and CONCLUSION

4.1 Breath interpretation

The ^{13}C carbon dioxide metabolized from labeled fructose appears in the breath more than that from glucose. Both labeled molecules are metabolized which is a fate of both of these molecules and there are other fates which were measured [8]. The MRI and blood measures attempt to capture other intermediates and the metabolism. However, more carbon dioxide is formed as those products are broken down. Eventually, a steady state is metabolism will be reached since only so much catabolism occurs which creates carbon dioxide.

4.2 Liver interpretation

Some studies do address the biases and corrections that are inherent with the quantification of the liver [9]. Liver volume loss from weight loss studies are a comparison of a pre and post diet; fluctuations in volume can be reported from a gain to a loss of about 5% [10]. Changes in liver volume of about 10% to 40% can be seen in dietary and pre-operative studies depending on intervention [11]. Liver volumes observed in the participant pool fluctuated about 5% or more over the course of the day. Holistically observing the fat in an individual requires many different types of measurements at specific times. The liver volume and fat fraction can indicate different narratives of what is occurring in the body over time; fat content does combine this data to capture a more complete picture of fat in the liver. Liver volume decreases which could mean a loss of fat or glycogen, however, fat fraction increases which could be interpreted as an increase of fat. Fat content indicates the amount of fat and allows interpretation regardless of any other changes occurring in water or volume. Since the liver is highly vascularized, triglycerides in the liver are observed in the PDFF, ^{1}H MRS, and ^{13}C MRS scans.

4.3 Blood interpretation

By taking blood measures, fat that newly forms can be observed in the blood which is important to understand that a person may lack fat in their liver, but it could be circulating in their bloodstream.

4.4 Fat export versus storage interpretation

Fat can be: metabolized, exported into the blood, or stored in the liver, in this study's context. Choline does intertwine with lipoprotein vesicle formation which is the mechanism by which fat molecules can be exported. As a low choline diet results in liver damage and steatosis as a result of being unable to export fats, a balance of choline in the diet would be important to maintaining homeostasis between fat storage and fat export [12]. Each has an associated risk, therefore, managing intake would be important in determining health outcomes. Currently, it is unknown how to quantify an amount of choline needed to cause a specific effect and whether it is threshold-based or dose-dependent. With more research, the claim that high choline fraction is associated with less fat stored in the liver and that low choline fraction is associated with less export of fats into the blood could be substantiated.

4.5 Segmentation

A semi-automatic approach needs to be employed since not enough data has been acquired for a fully automatic method; a semi-automatic method also results in less variability in quantification [13]. The segmentation struggles with organs that appear similarly in an MRI, thus creating larger compartments as it assumes all the pixels are part of the same structure. Since the final segmentations are modified, they do not suffer from this effect. These results indicate that it is important to use the automatic section of this method to identify the clear borders of the liver and surrounding tissue and utilize manual segmentation to adjust organ interfaces.

4.6 Peak quantification of ^{13}C metabolites

The approach to quantify and normalize the ^{13}C data visually flattened the baseline and allowed for comparison between the timepoints both in raw values and change over time. Differences in ^{13}C metabolites to be quantified provides insight into how much of this isotope is retained in the liver in that timepoint. However, the new baseline is not perfect. It does standardize data, but it could be affecting the area under the peaks for other metabolites away from the CH_2 peak. It is also possible that the constraints for the amplitude or location of the peaks may have been too rigid for some spectra and too relaxed for others. An increase in ^{13}C glycerol being associated with an increase in ^{13}C CH_2 lipids can be due to the formation of triglycerides from these independent structures. For triglycerides to form, both of these compounds need to be present and a surplus of one compound could stimulate the production of the other to continue the storage of lipids in the liver [14]. In this method, glycerol was quantified, but it was not noted which pathway it had undergone to form glycerol; some processes are more direct whereas others are more time-consuming and have more intermediates [15]. Differences in these processes can result in additional variation due to multiple reactions potentially driving change in glycerol production over time.

4.7 Limitations

The study consists of only six volunteers which is a low number of participants to recruit. However, the power is strengthened by the number of measurements taken since each participant is tested nine times including baseline and is studied on two days. While there is some dependence on some measures as they are conducted on the same individual, each MRI exam is independent since the participant is repositioned in the scanner every time. HOMA-IR refers to a measure of insulin resistance and sensitivity and by having different levels across the subjects,

metabolic pathways are impacted. Many pathways for consumed food are insulin dependent and the inability to respond or low sensitivity will elicit different responses [16].

To accurately gauge fructose and glucose metabolism in the body, a day where only fructose and another with glucose respectively are served would provide a picture of what occurs in the body. In this study, the shakes consumed were composed of equal parts fructose and glucose but one of the two was labeled. However, a mixture of fructose and glucose is more representative of a typical diet; solely consuming one sugar may lead to a different metabolism, for example, more fructose may be converted into glucose.

Differences among the individuals does create variation in the data. Therefore, studying more individuals would create more confidence in the results obtained from the study. As many of these measures are continuous values, adding more subjects would add confidence in the values obtained and would strengthen correlation between metabolic events. Also, managing metabolic need impacts the study. Individuals who were fasted and received too many nutrients will elicit a different response than those who receive too few nutrients. Also, the timing of their effects may not accurately represent their normal metabolic state under nutritionally deprived or overfed conditions.

4.8 Conclusion

In conclusion, this study of metabolic measures in a fasted state, then hourly, after hourly feeding, demonstrated some metabolic changes during the day: (1) fructose was metabolized into carbon dioxide significantly more than glucose; (2) liver volume was significantly lower at 2 and 3 hours post feeding, then increased during the remainder of the day; (3) fat content in the liver was significantly lower 3 and 4 hours post feeding; (4) high choline individuals export more lipids into the blood; (5) low choline individuals store more fat in their liver.

The fact that liver volume significantly decreased when liver fat fraction significantly increased while the fat content remained stable suggests that the increase in fat fraction was due to the loss of water. Thus, it is recommended that both liver volume and fat fraction are determined before interpreting changes in fat fraction.

This study also demonstrated a number of differences among subjects, particularly in timing of changes during the course of the day, which may be due to different metabolic needs or other differences in metabolism. Further studies are required to elucidate these effects.

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